Structural characterization of thermoresistant enteroviruses

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ISFEV 2018

Introduction

Temperature and thermostability are important in:

Food consumption

Fat content

Vaccination

$MgCl_2$

Environmental persistence

Sediment/adsorption

The matrix and the virus significantly influence thermostablity

Introduction

Article

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Variability in Disinfection Resistance between Currently Circulating *Enterovirus B* Serotypes and Strains

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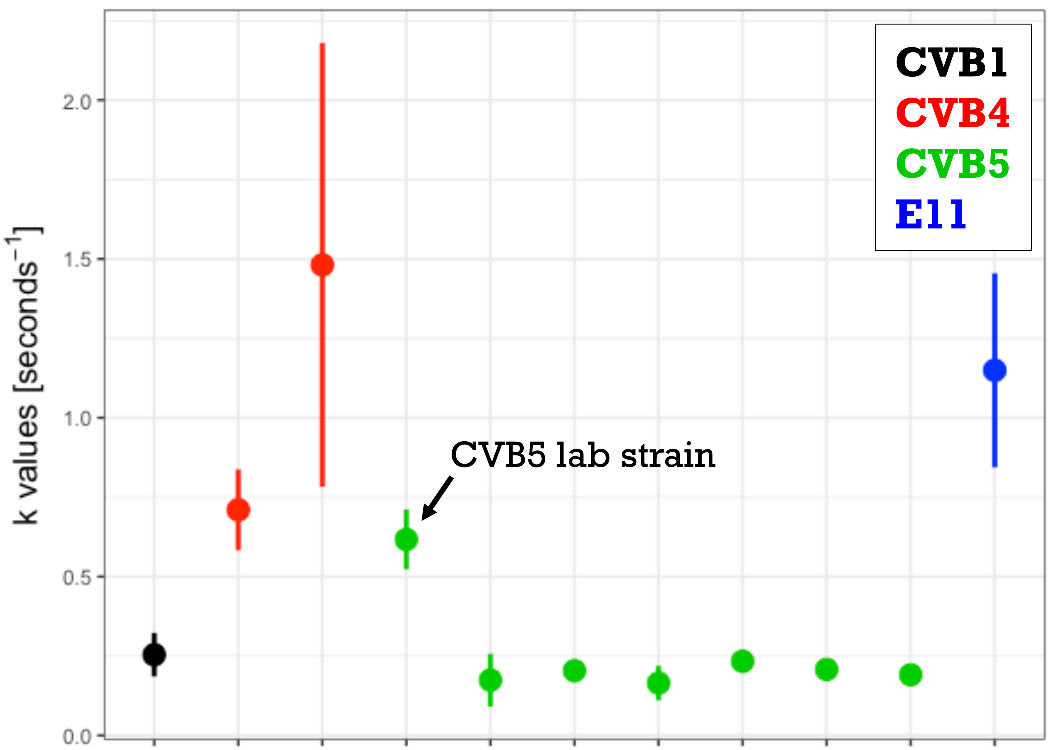
Thermal Inactivation at 55°C

k values



1. CVB1 and CVB5 isolates are more thermostable than CVB4 isolates and E11

2. CVB5 laboratory strain less thermostable than CVB5 isolates

Same buffer used !

Introduction

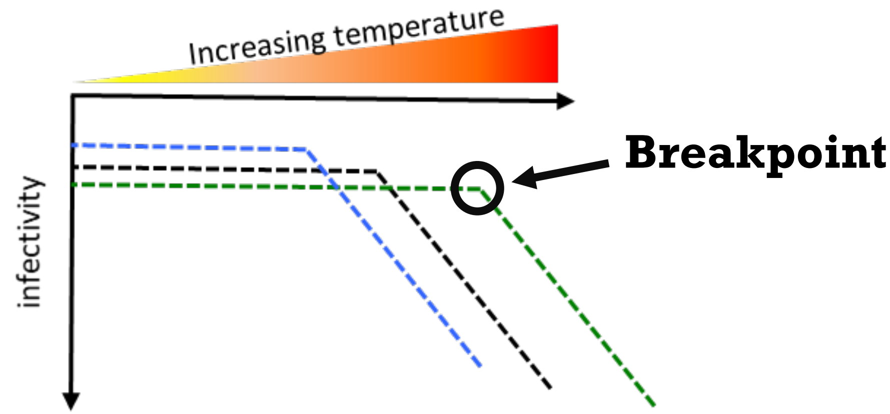
Goals:

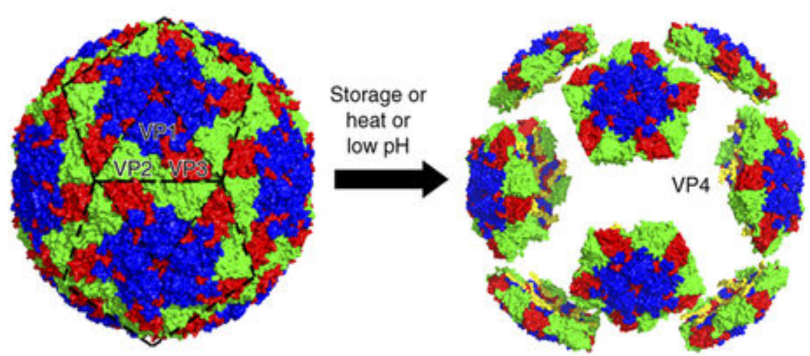
1. Identify the mechanisms of virus inactivation during thermal inactivation

2. Characterize structural features that enhance thermostability

Approach:

1. *Thermal-shift assay to determine the breakpoint*
2. *Capsid modeling*
3. *High and Low temperature*
4. *Heat adaptation*

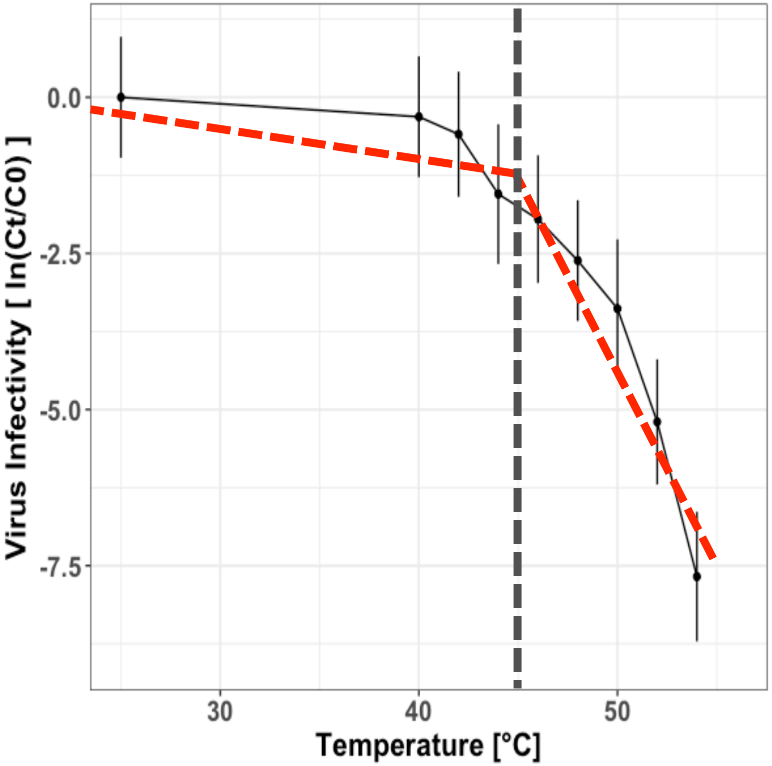


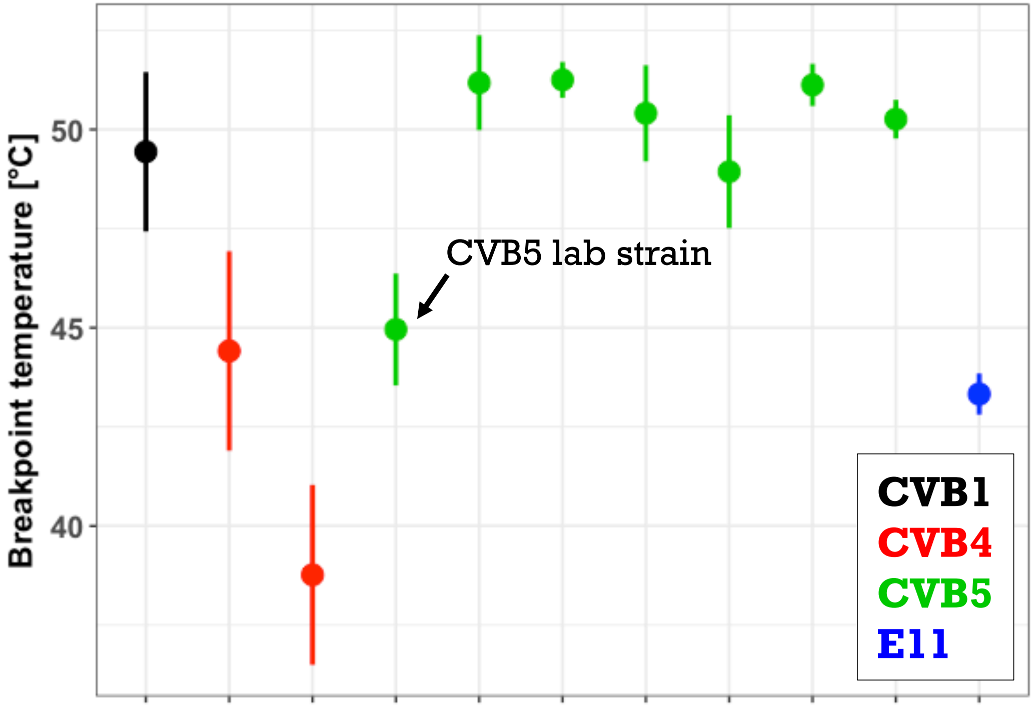


Kotecha et al. 2015

Results

Breakpoint



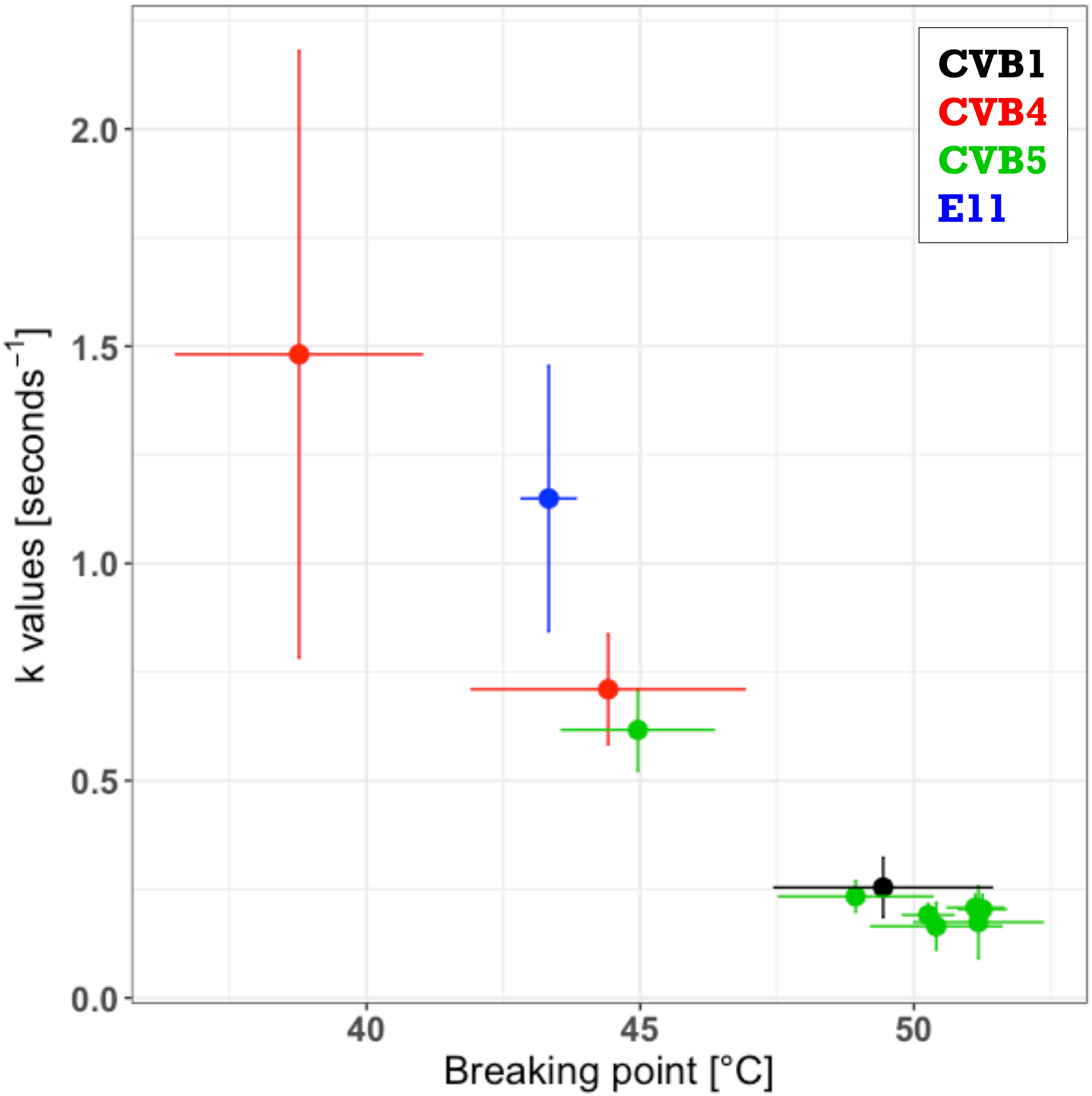


→ **CVB1 and CVB5 strains have high breakpoints**

→ **E11 and CVB4 strains have low breakpoint**

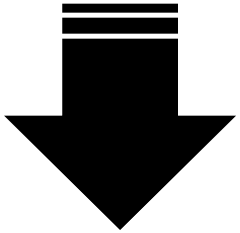
Results

Correlation: Breakpoint with k values



Break point is indicative of thermal inactivation kinetics at 55°C.

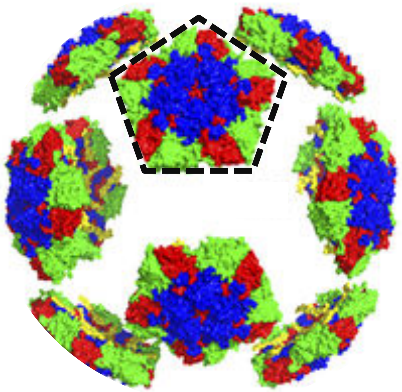
But why does thermostability differ?



CAPSID MODELING

Results

Deeper insights on the capsid structure of the isolates



→ 3D modeling of the capsid isolates using CVB3 as scaffold

→ Analysis of different parameters:

Disulfid bonds

Hydrogen bonds

Hydrophobic core pockets

Salt bridges

Electrostatic interactions

Structure

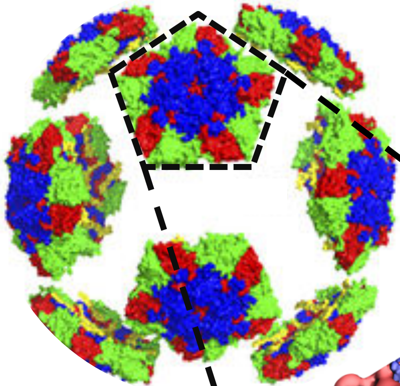
Identification of the Structural Basis of Thermal Lability of a Virus Provides a Rationale for Improved Vaccines

Verónica Rincón,[1] Alicia Rodríguez-Huete,[1] Silvia López-Argüello,[1] Beatriz Ibarra-Molero,[2] Jose M. Sanchez-Ruiz,[2] Michiel M. Harmsen,[3] and Mauricio G. Mateu[1,*]

1. **Negatively charged residues exert electrostatic repulsion between capsid subunits**
2. **Compensated by dipole-dipole**

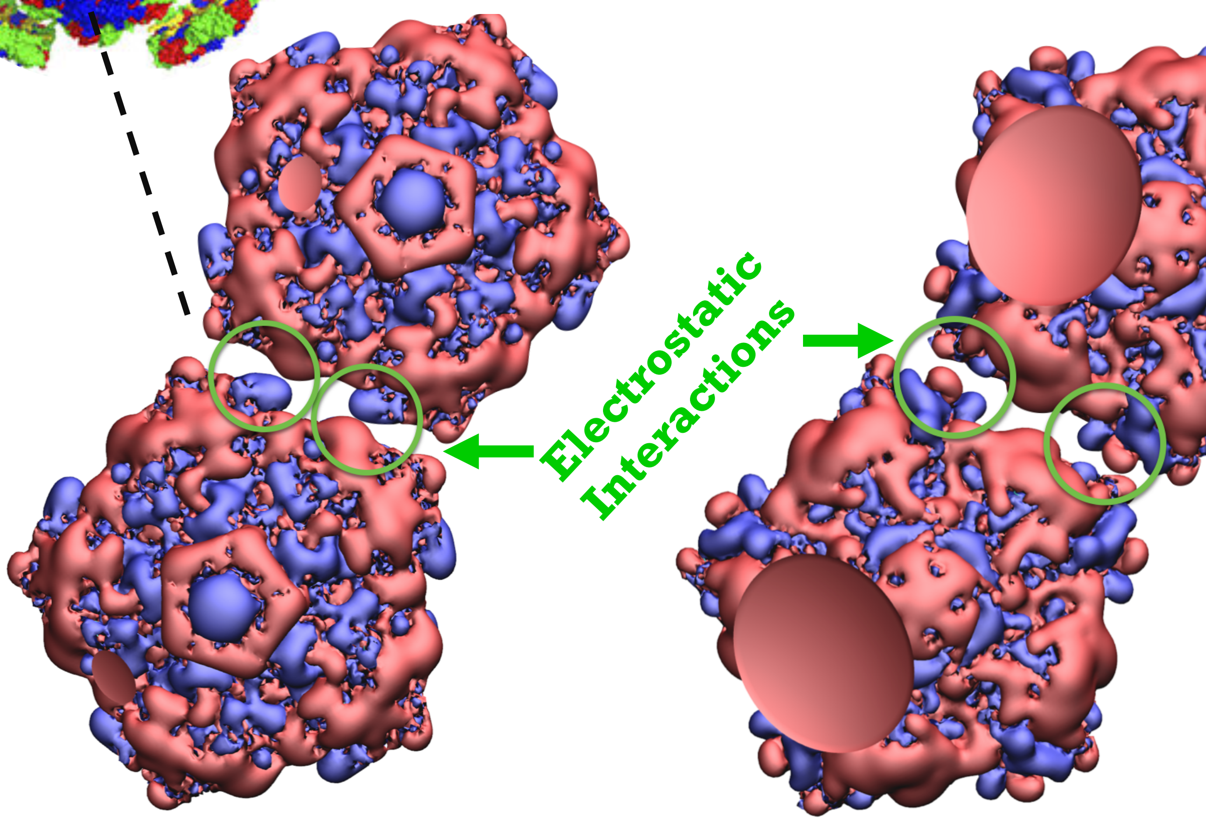
Results

Deeper insights on the capsid structure of the isolates



→ 3D modeling of the capsid isolates using CVB3 as scaffold

POSITIVE and **NEGATIVE** electrostatic potentials



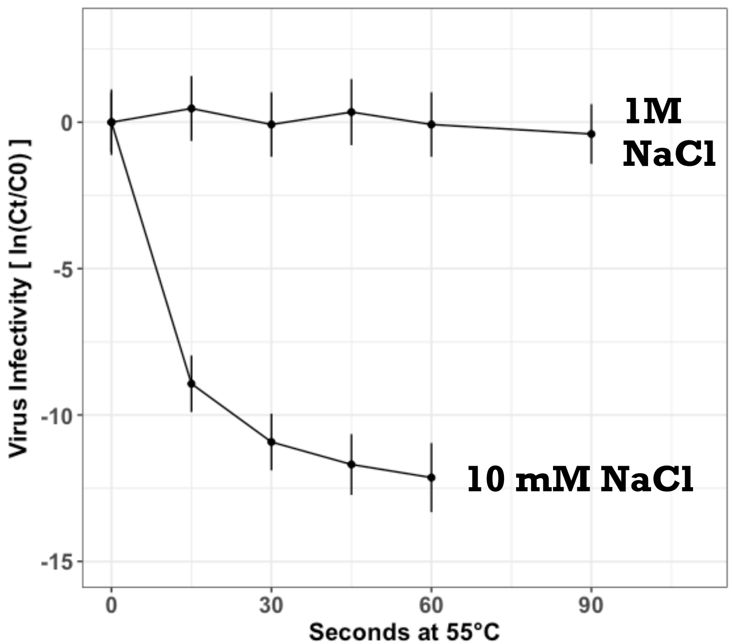
CVB5 and CVB1 **CVB4**

Salt

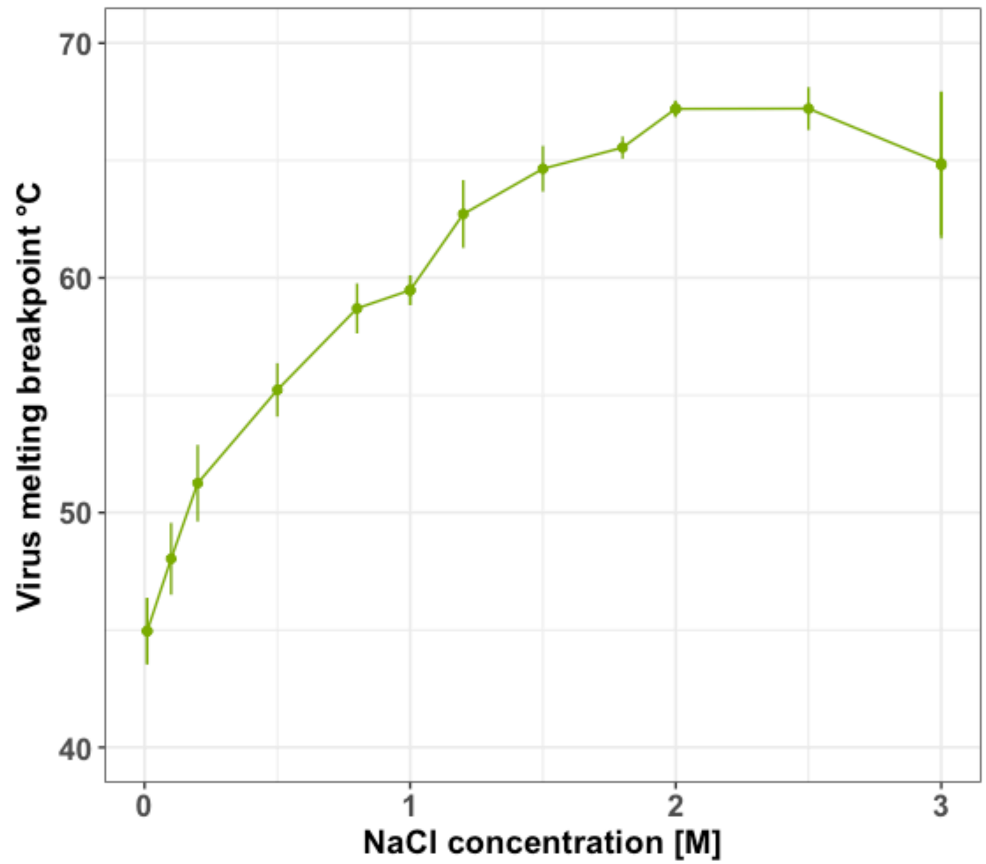
Results

Effect of salt on thermostability

55° C



Thermal-shift assay with
increasing salt concentration

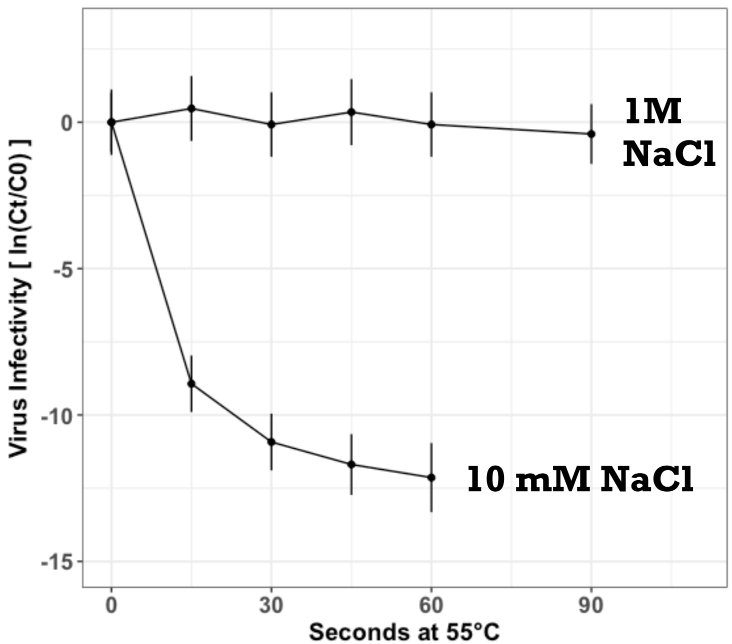


1. **Highly protective effect of salt**
2. **But what about breakpoints ?**

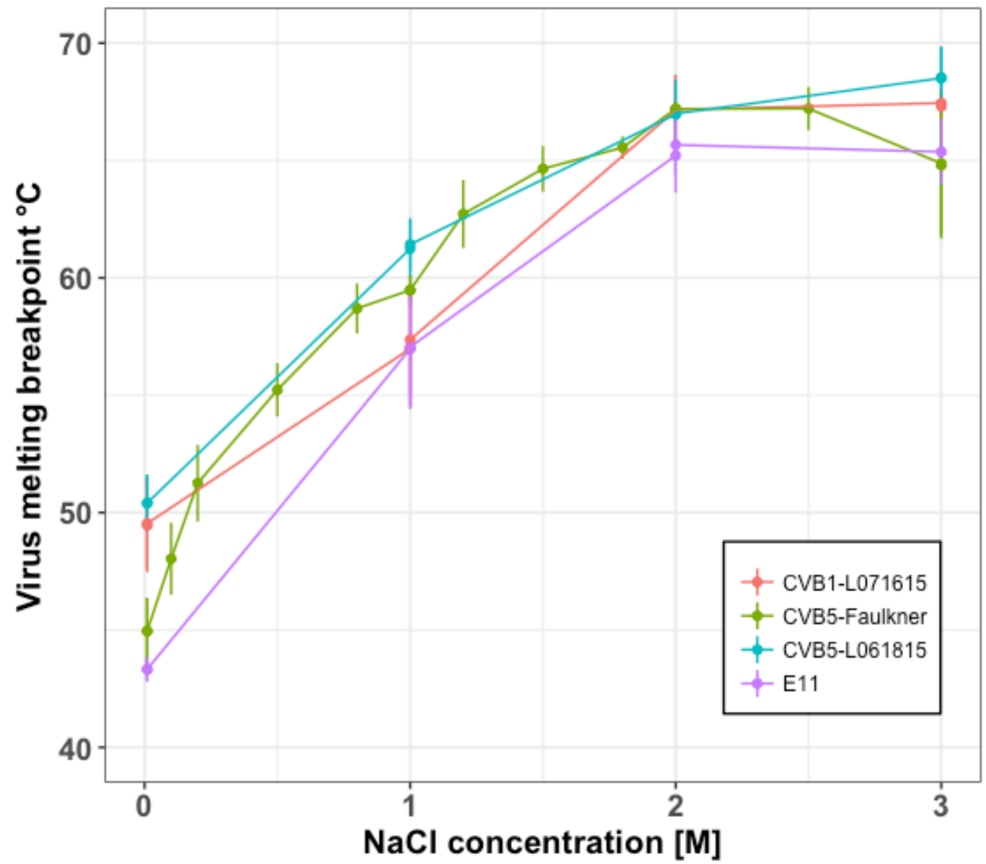
Results

Effect of salt on thermostability

55° C



Thermal-shift assay with increasing salt concentration

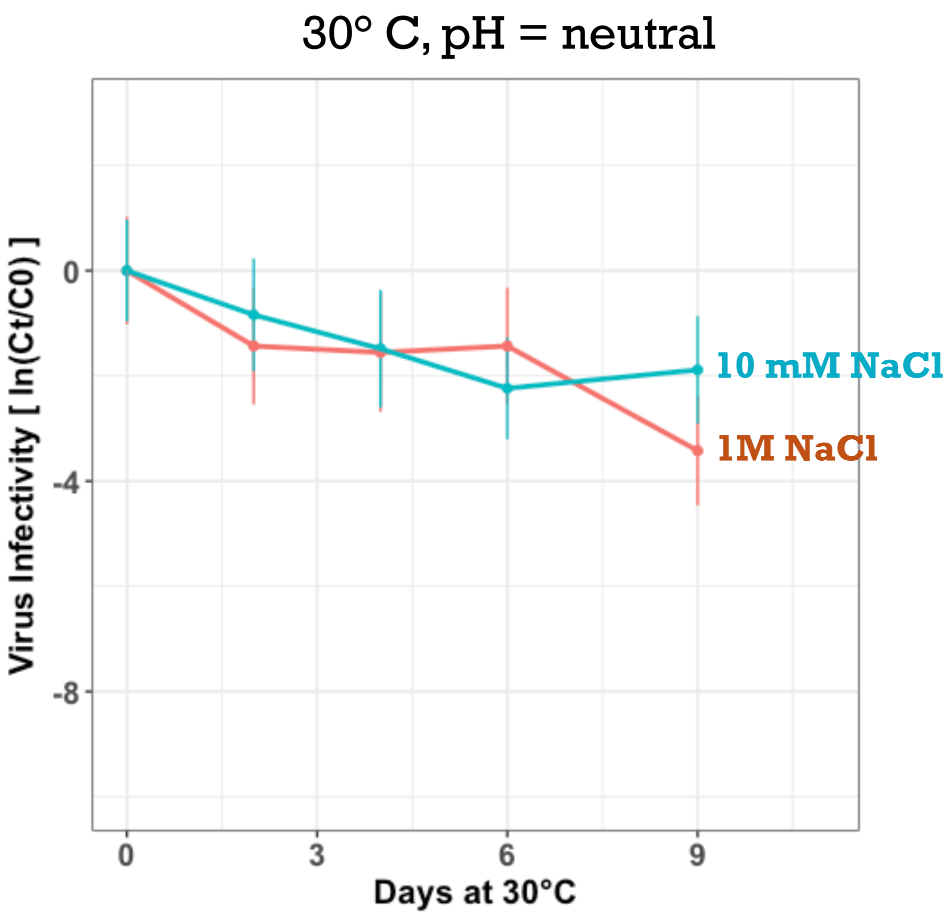


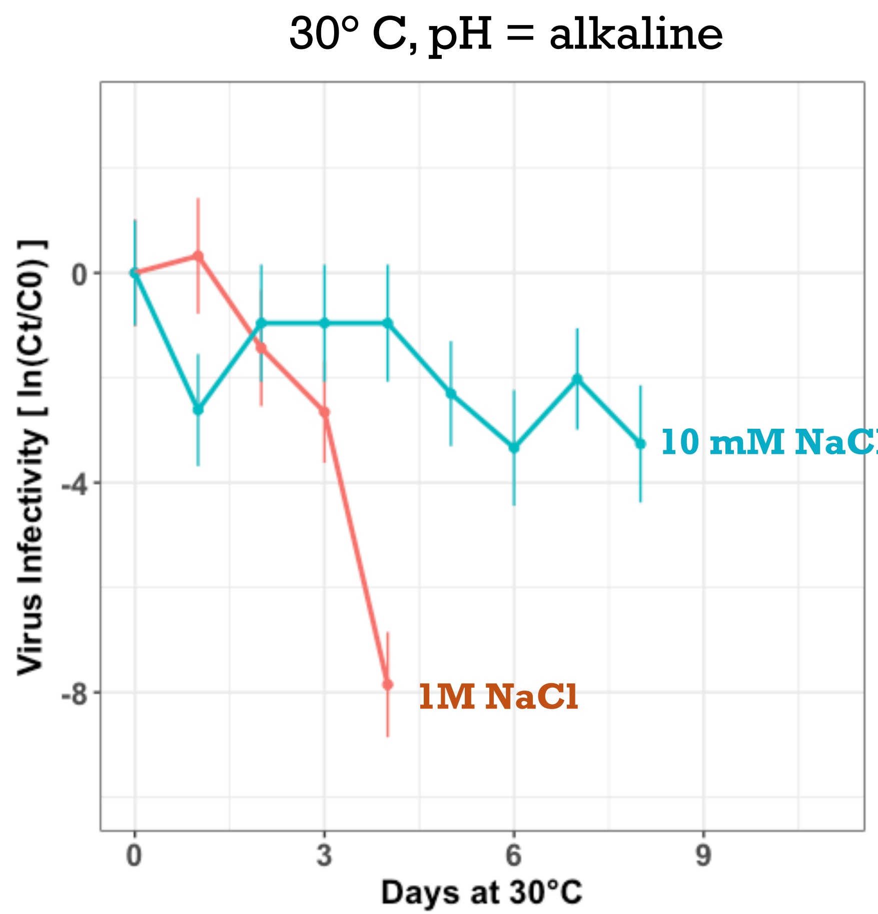
1. Highly protective effect of salt
2. But what about breakpoints ?

→ **The breakpoints can be shifted up by 20 degrees**

Results

Effect of salt on thermostability





1. Effect of salt at 30° C only at alkaline pH
2. Genome damage occurs, but does not correlate with infectivity loss

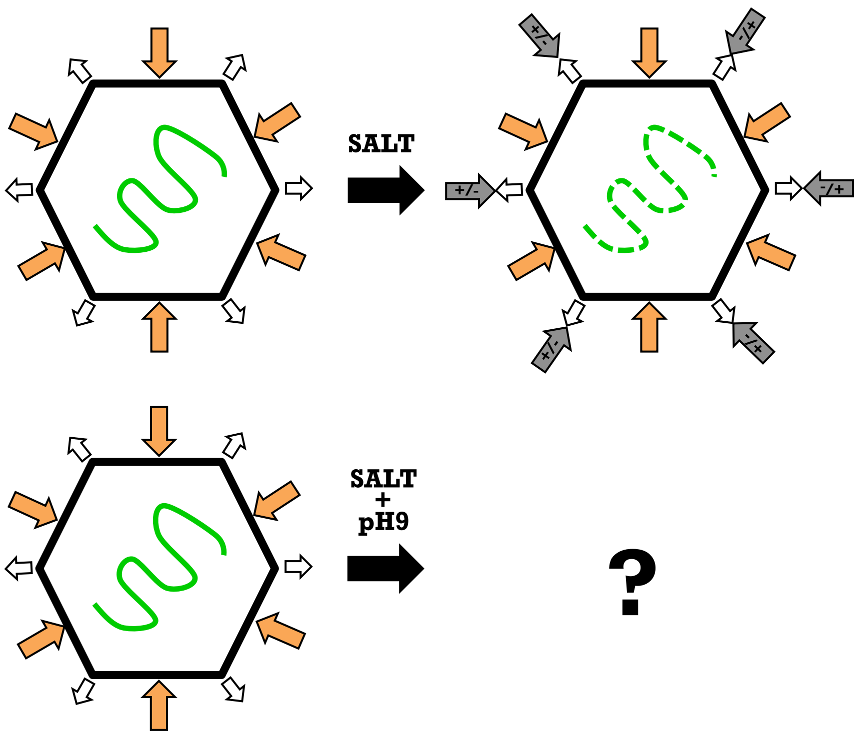
→ The inactivation mechanisms are not the same at 55 and 30° C

Results

Proposed mechanisms

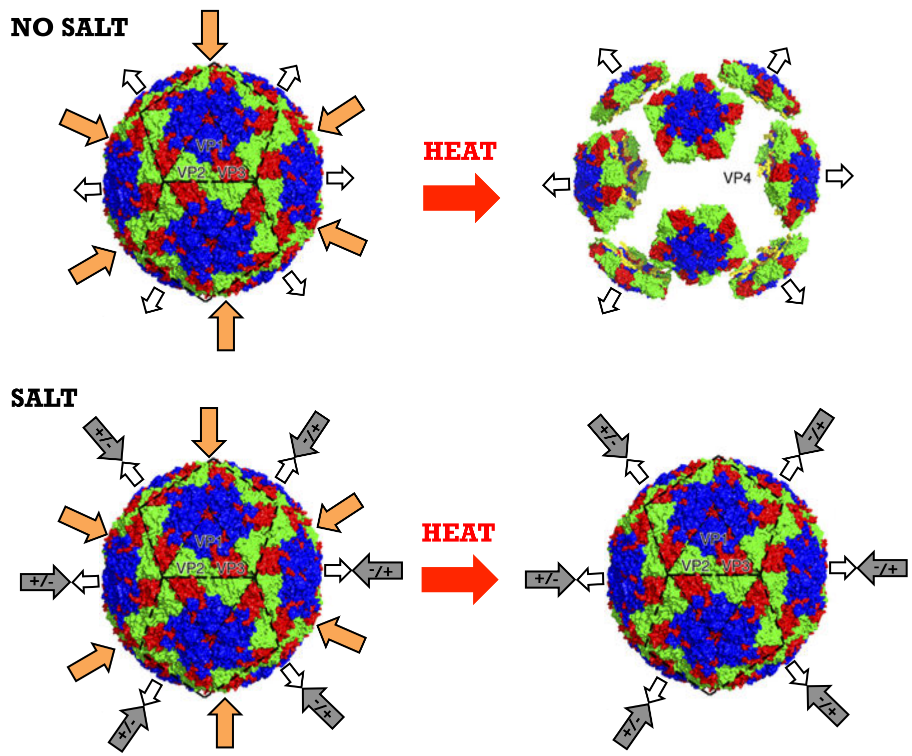
At 30° C

1. Genome degradation occurs but does not correlate to infectivity loss
2. Effect of salt only at alkaline pH



At 55° C

1. Capsid disintegration dominates
2. Salt shields repulsive interactions and thereby protects capsid



Results

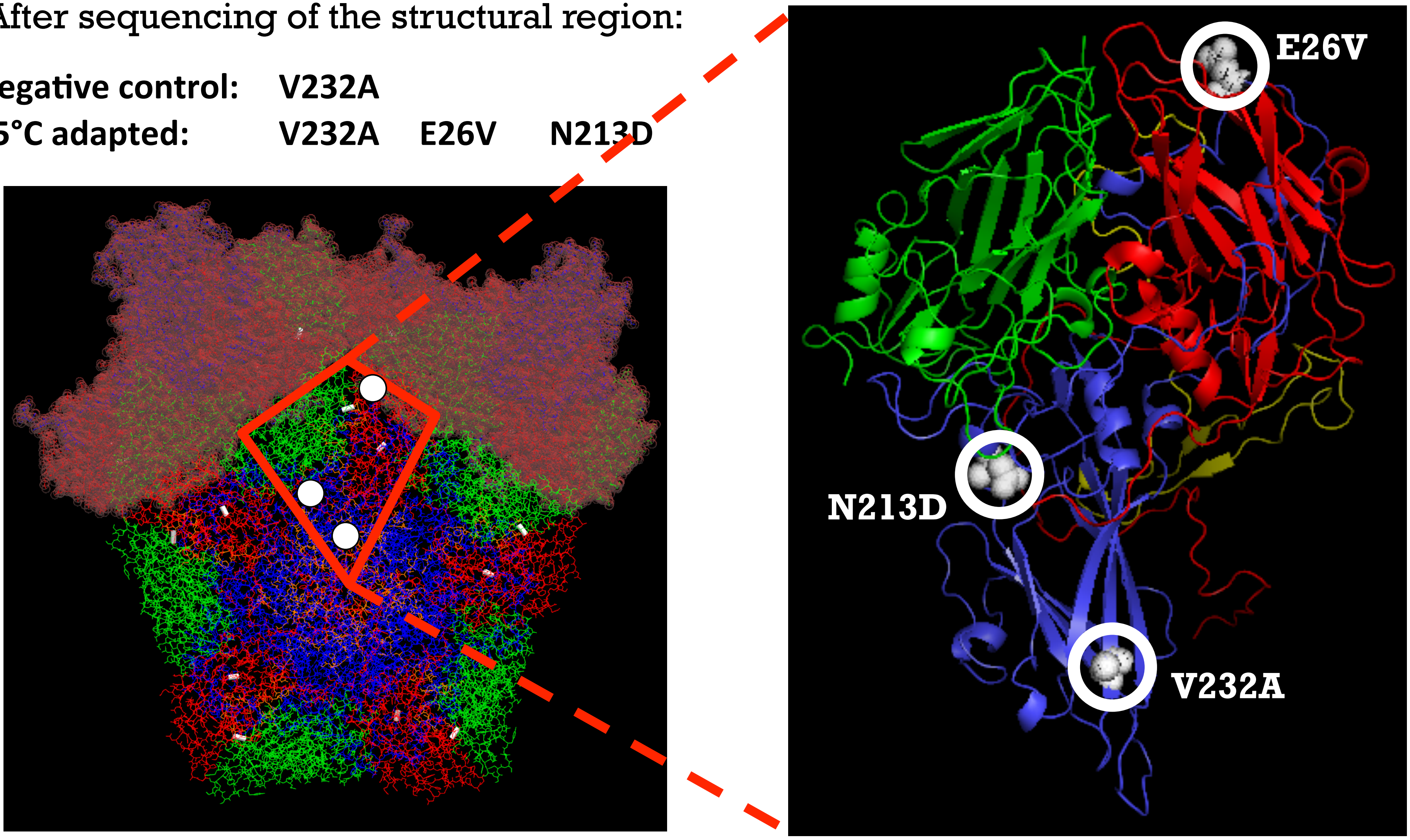
Adaptation of thermoresistant CVB5

→ They adapted !

After sequencing of the structural region:

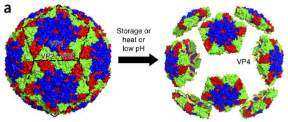
Negative control:	**V232A**		
55°C adapted:	**V232A**	**E26V**	**N213D**

E26V Mutation located at the interface of the pentameres



Summary and Conclusion

1. Identify the mechanisms of virus inactivation during heat treatment

- For high temperature inactivation:  AND protective effect of salt

- For low temperature inactivation: Still under investigation

2. Characterize structural features that enhance thermostability

- Electrostatics interaction within the pentameric interface

→ *Further analysis on going to see how those electrostatics change when the salt concentration is increased*

3. Identify mutations associated to thermal stability

- Electrostatics interactions within the pentameric interface

→ *Amino acid change from neutral to negative side chain charge*

Even simple matrices can have a strong capsid stabilizing effect

Thermal inactivation can be less effective than expected

Acknowledgments

Thank you

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Virginie Bachmann

Jade Brandani   Karine Maritz

FNSNF

SWISS NATIONAL SCIENCE FOUNDATION

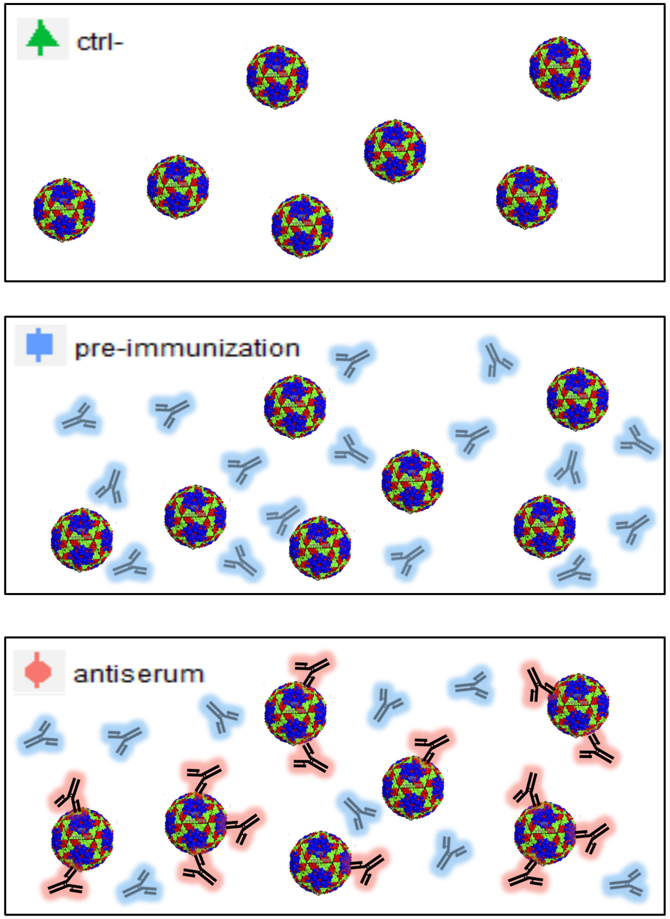
EPFL

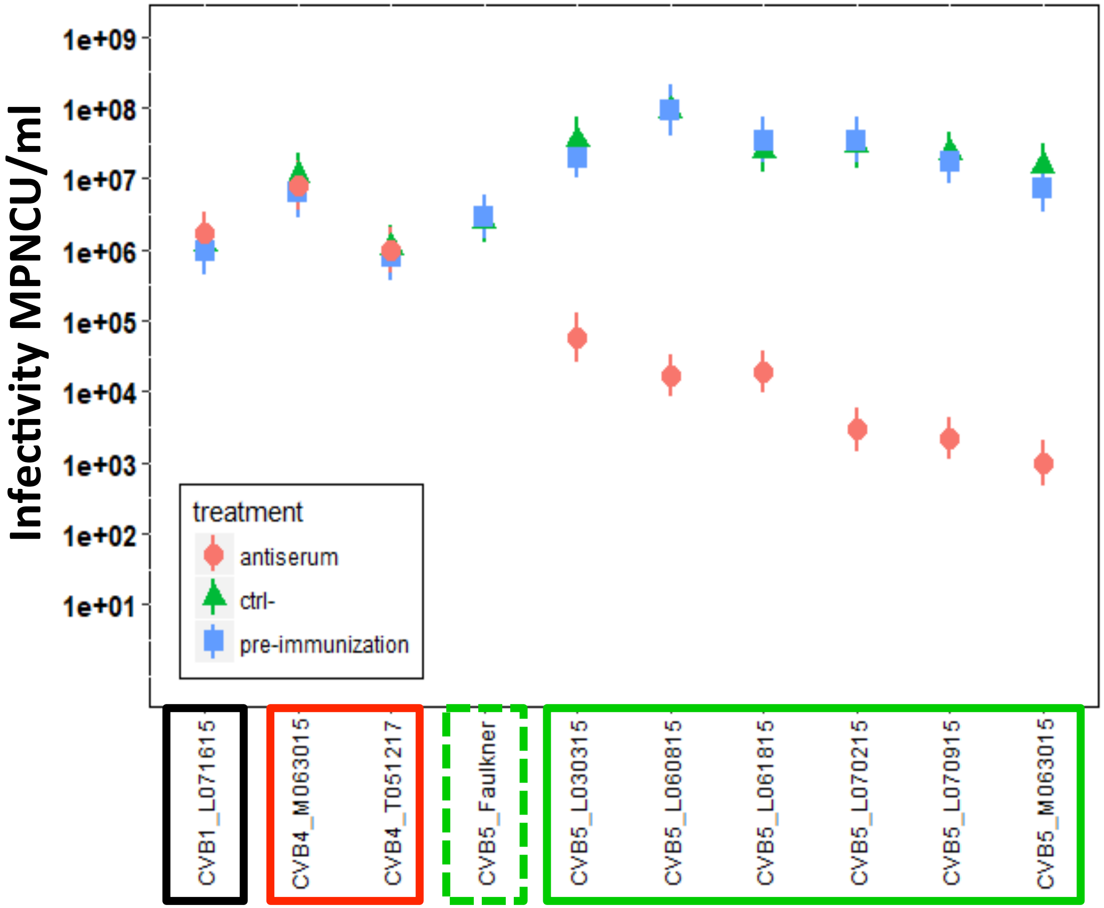
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Results

First insights on the capsid structure of the isolates

Antigenic divergence assay

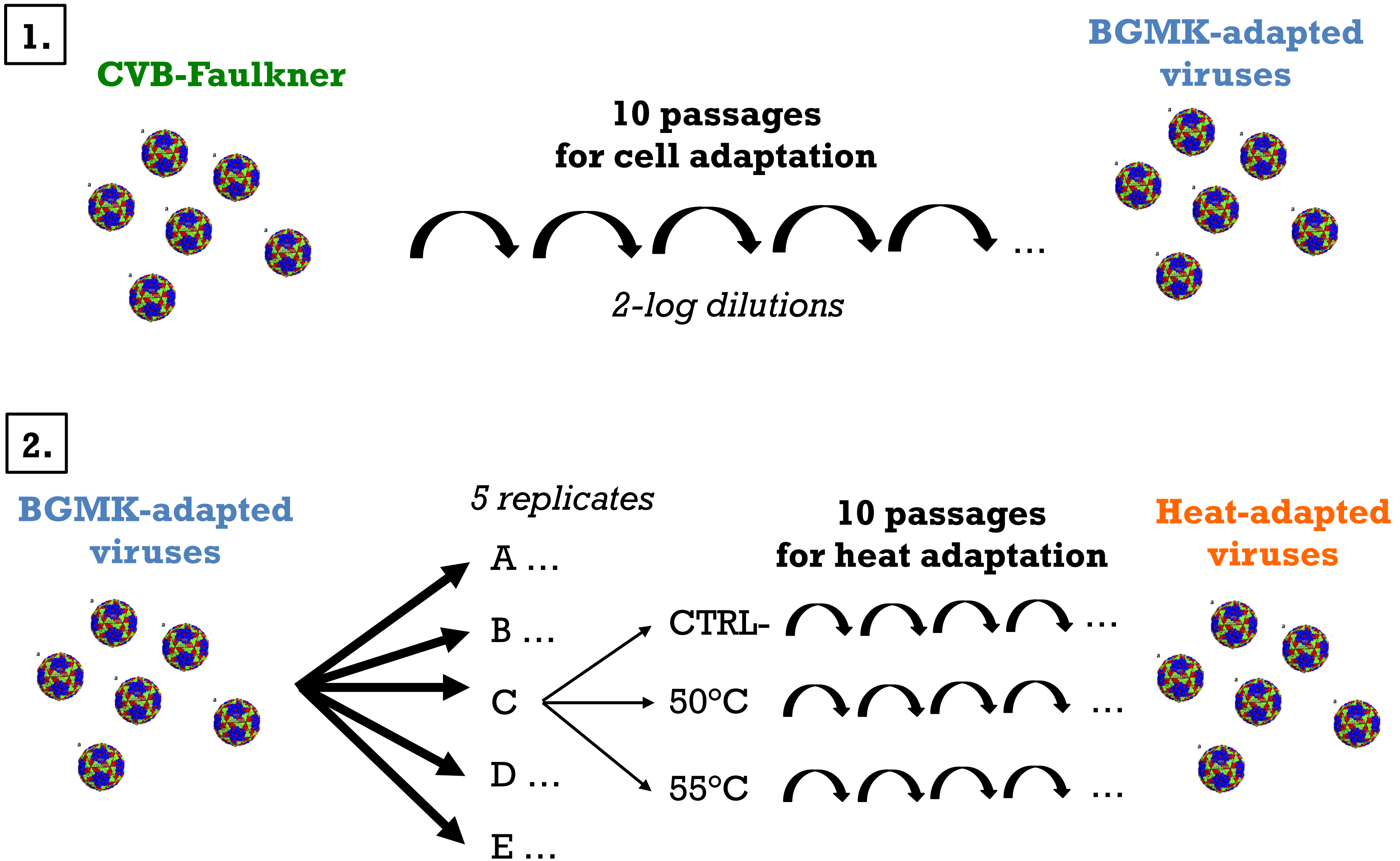




→ **The CVB5 isolates have only a partial inactivation**

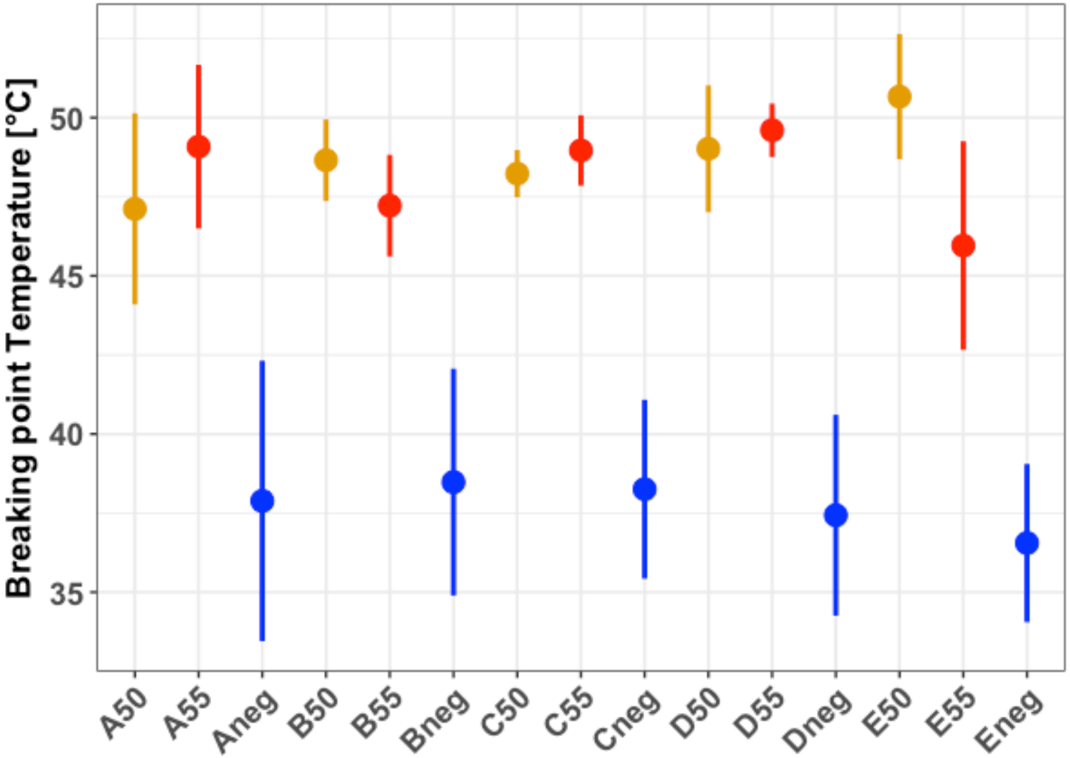
Results

Insights on heat adapted viruses

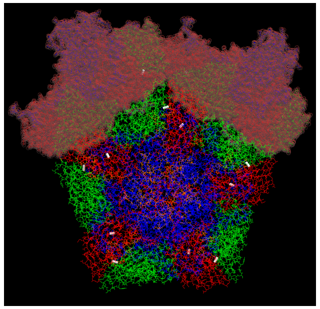


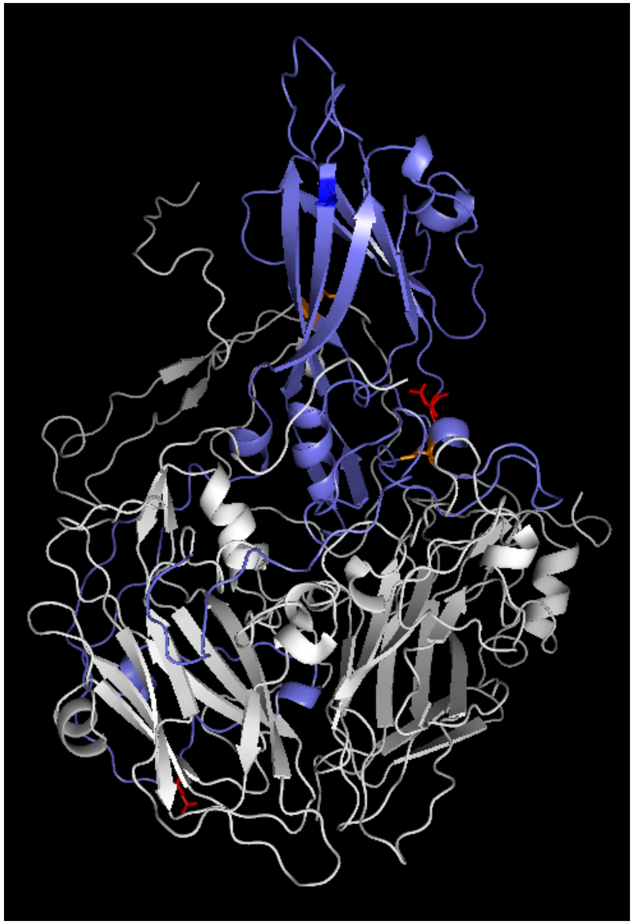
Results

Thermal-shift assay



50°C adapted
55°C adapted
CTRL-





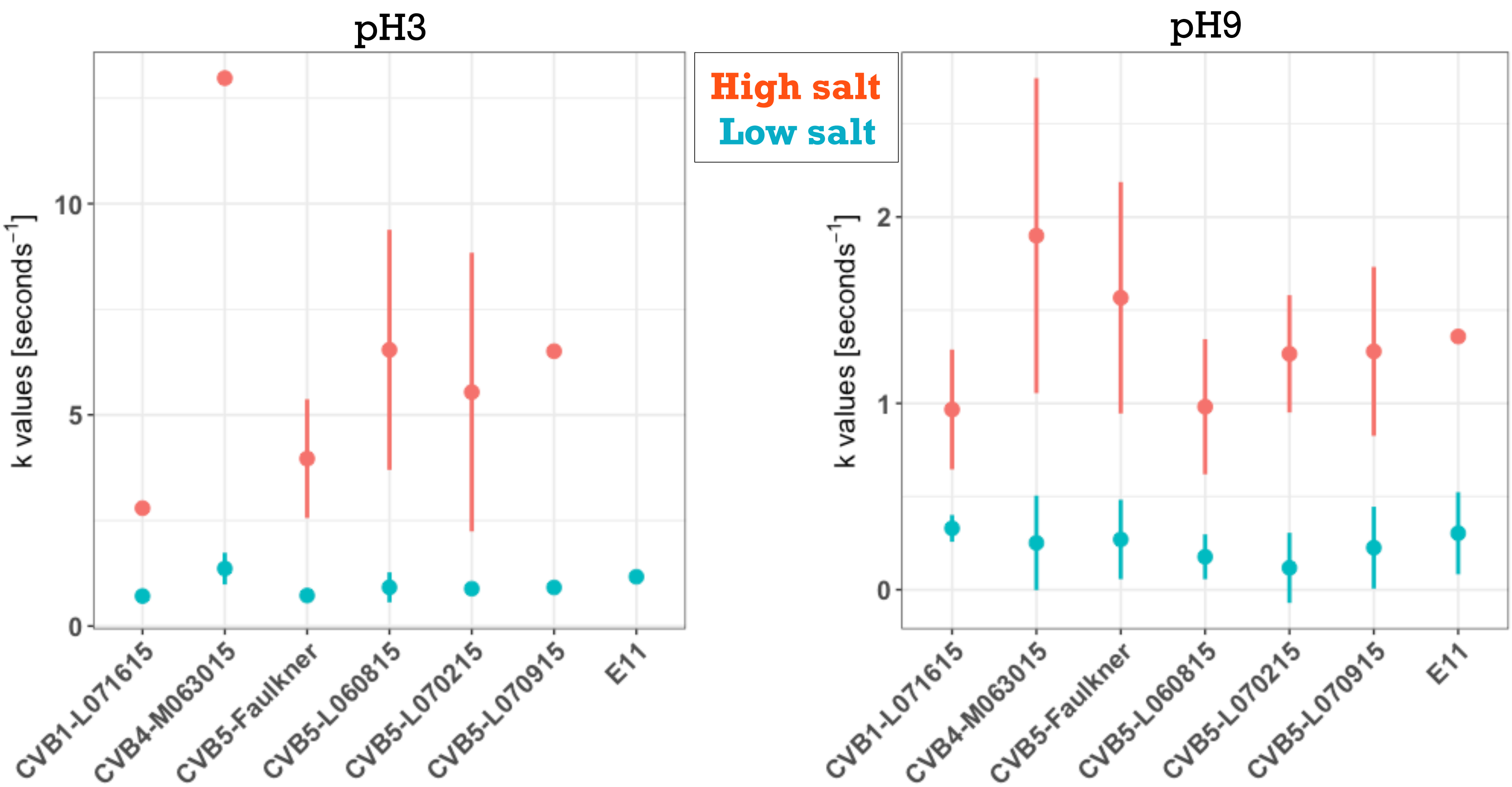
→ They adapted !

After sequencing of the structural region:

Model 1:	Negative control	V232A		
Model 2:	50°C adapted virus	V232A	I209F	
Model 3:	50°C adapted virus	V232A	M180V	
Model 4:	55°C adapted virus	V232A	E26V	N213D

Results

Effect of salt and pH on thermostability at 30°C

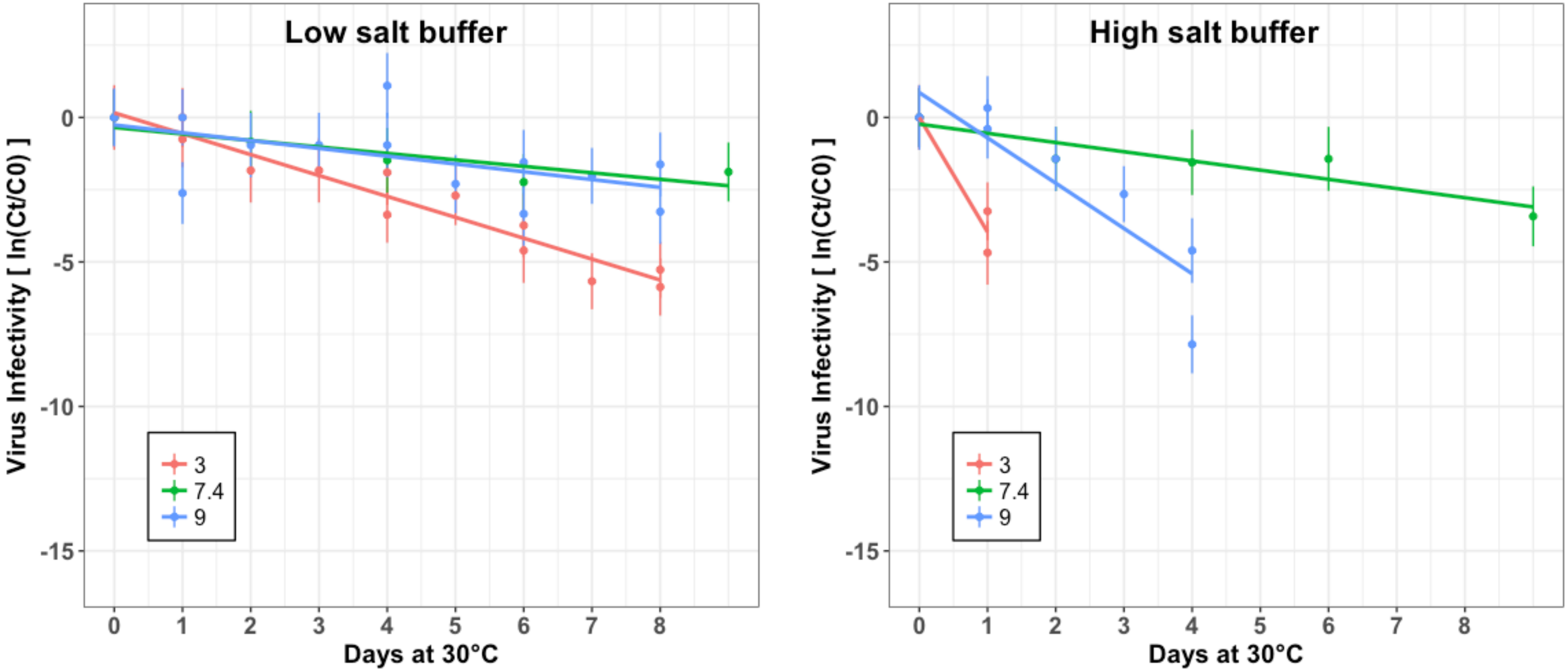


→ CVB4 appears as the most salt/pH-sensitive, and CVB1 the least

Results

Effect of salt on thermostability

30° C



No effect of salt at 30° C

→ The inactivation mechanisms are not the same at 55 and 30° C !